Presence of two forms of apolipoprotein B in patients with dyslipoproteinemia

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Abstract Current information suggests that the major forms of the human B apolipoproteins, B-100 and B-48, are under separate genetic control and are synthesized by the liver and intestine, respectively. The apolipoprotein B composition of plasma lipoproteins has been determined in order to gain insight into the metabolic defects in patients with dyslipoproteinemias. Patients with type I and type V hyperlipoproteinemia can be separated into two groups based on apolipoprotein composition and triglyceride concentration. The first group had markedly elevated plasma triglycerides with B-48 in the 1.006 g/ml density fraction and only B-100 within IDL and LDL. The second group had plasma triglycerides < 1200 mg % and only B-100 in all density fractions. Patients with type III hyperlipoproteinemia had B-48 in only the density < 1.006 g/ml with B-100 in IDL and LDL; the type III hyperlipoproteinemic patient with apolipoprotein E deficiency, however, had B-48 in density < 1.006 g/ml fraction, IDL, and LDL. Patients with type IIa, IIb, and IV hyperlipoproteinemia had only B-100 in all density fractions. These combined results are interpreted as indicating that B-48 is associated with triglyceride-rich lipoproteins synthesized by the intestine and that patients with phenotypes I, III, and V have defects in chylomicron remnant metabolism. In addition, in patients with types I and V hyperlipoproteinemia, mild hypertriglyceridemia appears to be associated with lipoprotein particles of liver origin.—**Meng, M. S., R. E. Gregg, E. J. Schaefer, J. M. Hoeg, and H. B. Brewer, Jr.** Presence of two forms of apolipoprotein B in patients with dyslipoproteinemia. *J. Lipid Res.* 1983. **24:** 803–809.

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During the last decade several laboratories have studied the physicochemical properties of apolipoprotein B, however, this apolipoprotein has been particularly difficult to isolate and characterize owing to its insolubility in aqueous buffer in the absence of denaturants or detergents (1–5). Recently, studies have demonstrated structural heterogeneity of apoB by NaDodSO₄ electrophoresis (6, 7). In the rat (6) and the human (7), two major forms of apoB with different apparent molecular weights by NaDodSO₄ electrophoresis have been identified. In the human these two forms have been designated as B-100 and B-48 (7), and in both man and rat the larger apparent molecular weight form of apoB has been proposed to be synthesized primarily in the liver (7, 8). B-48 is present on triglyceride-rich lipoproteins in lymph and plasma and has been proposed to be synthesized by the intestine in man (7), and by the intestine as well as the liver in the rat (8). In normal man, B-48 appears to be removed from the plasma with chylomicron remnants and virtually no B-48 can be isolated within LDL (7). The major B apolipoprotein in human LDL is B-100, which is derived from catabolism of hepatic VLDL with initial conversion of the lipoprotein particle to IDL, and finally, LDL (9–12). B-100 on LDL interacts with a high affinity receptor on peripheral cells (13) and the liver (14–17), resulting in the cellular uptake of LDL. The metabolic heterogeneity of the two forms of apoB has been analyzed, and the lower molecular weight form of apoB was cleared more rapidly than the large molecular weight apoB in the rat (18) and in humans (19).

Four human dyslipoproteinemias have been identified with abnormally low plasma concentrations of B-100 and B-48. Abetalipoproteinemia is characterized by the absence of normal chylomicrons, VLDL, IDL, and LDL, and no detectable plasma B-100 or B-48 (20). Patients with homozygous hypobetalipoproteinemia are also deficient in apoB-containing plasma lipoproteins, however, the clinical symptoms of this disease are somewhat ameliorated (20). A third disease has been described which is characterized by severe hypobetalipoproteinemia with normal absorption of triglycerides (21). The latest disorder associated with the B apolipoproteins to be described is normotriglyceridemic abetalipoproteinemia (22). This disorder is characterized by the presence of B-48, the absence of B-100, and normal absorption of dietary lipids. Based on these and other data, it has been proposed that B-48 and B-100

Abbreviations: apo, apolipoprotein; NaDodSO₄, sodium dodecyl sulfate.

are under separate genetic control, and that in normal man no significant quantity of plasma apoB within LDL is derived from the metabolism of chylomicron remnants. In order to gain insights into the importance of defects in metabolism of plasma lipoproteins containing B-100 and B-48 in patients with dyslipoproteinemia, we have analyzed the plasma lipoproteins for the presence of B-48 and B-100 in fasting hyperlipoproteinemia patients with phenotypes I to V (21), and several rare genetic diseases including Wolman's disease, lecithin cholesterol acyltransferase deficiency, Tangier disease, betasitosterolemia, and Niemann-Pick disease.

MATERIALS AND METHODS

Patient selection

Patients selected for study attended the clinic of the Molecular Disease Branch at the National Institutes of Health. Patients were instructed to discontinue lipid-lowering drugs, and to consume a regular diet for 4 weeks prior to blood sampling. For the purpose of this study, the lipoprotein phenotype was ascertained at the time of analysis and in all patients was virtually identical to that determined on previous analyses. The following criteria were employed in the classification of the patients with hyperlipoproteinemia (23).

1) Type I (N = 10): significant hypertriglyceridemia associated with fasting chylomicrons, post-heparin lipoprotein lipase deficiency (N = 9) (24), or deficiency of the apoC-II cofactor for lipoprotein lipase (N = 1) (25).

2) Type IIa (N = 10): LDL cholesterol greater than the 90th percentile for age and sex (26), plasma triglycerides and VLDL-cholesterol levels within the normal range, and increased beta-lipoproteins on paper electrophoresis. Three patients with homozygous familial hypercholesterolemia (type IIa phenotype) were also analyzed. These patients had LDL cholesterol levels greater than 800 mg %, and were shown to have a defect in LDL receptor binding by analysis of fibroblasts grown in tissue culture (13).

3) Type IIb (N = 10): VLDL cholesterol and LDL cholesterol greater than the 90th percentile and increased beta and pre-beta lipoproteins on paper electrophoresis (26).

4) Type III (N = 10): VLDL cholesterol above the 90th percentile with a VLDL cholesterol to plasma triglyceride ratio greater than 0.3 and a broad beta band on paper electrophoresis (27, 28).

5) Type IV (N = 10): Plasma triglycerides and VLDL cholesterol above the 90th percentile with normal LDL cholesterol level, normal VLDL cholesterol to plasma triglyceride ratio, and increased pre-beta-lipoproteins on paper electrophoresis (23, 26).

6) Type V (N = 10): hypertriglyceridemia associated with fasting chylomicronemia with no evidence of post-heparin lipoprotein lipase or apoC-II deficiency, and chylomicrons and increased pre-beta-lipoproteins on paper electrophoresis (23).

One patient each with Wolman's disease, lecithin cholesterol acyltransferase deficiency, Tangier disease, betasitosterolemia, and type B Niemann-Pick disease were analyzed.

Lipoprotein analysis

Blood for analysis was obtained in Na_2EDTA (1 mg/ml) containing sodium azide (0.05%) and Trasylol (1000 KIU/ml) after an overnight fast. Plasma lipoproteins were separated by ultracentrifugation into density < 1.006 g/ml, IDL (d 1.006–1.010 g/ml), LDL (d 1.019–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) (Beckman, 60 Ti rotor, 4°C) (29). HDL cholesterol was determined following dextran sulfate-magnesium precipitation of plasma or of the 1.006 g/ml infranatant when plasma triglycerides exceeded 400 mg/ml (30). Triglycerides and cholesterol were quantitated by automated enzymic methods on a Gilford System analyzer (Gilford Instrument Co., Oberlin, OH).

Isolated lipoprotein fractions were dialyzed against 0.01% EDTA, 0.05% sodium azide, pH 7.4, and delipidated by extraction with chloroform–methanol 2:1 (v/v) (31).

Analytical NaDodSO₄ gel electrophoresis

$NaDodSO_4$ gel electrophoresis, using 3% acrylamide, was performed by the method of Weber and Osborn (32). Delipidated proteins (20 μg–150 μg) dissolved in 50 mM Tris-HCl (pH 8.2) containing $NaDodSO_4$ (10 mg/mg of protein) were applied to the gels. Following electrophoresis the gels were fixed and stained in methanol–water–acetic acid 10:1:1 (v/v) containing 0.001% Coomassie brilliant blue R-250 (Bio-Rad Laboratories) for 1 hr and destained in 5% methanol–7.5% acetic acid. The ratio of B-100 and B-48 following $NaDodSO_4$ gel electrophoresis was determined on a Beckman Acta-III equipped with a gel scanner.

RESULTS

The presence of B-100 and B-48 was ascertained by $NaDodSO_4$ gel electrophoresis in density fractions < 1.006 g/ml, IDL, and LDL in all patients analyzed. The mobility of the B apolipoproteins is significantly influenced by protein load and a sample load of 20–40 μg protein gave discrete bands. In order to assure that

B-48 would be detected even at low concentrations, samples were also analyzed at a protein load of 150 μg. The majority of the electrophoretograms presented are at a sample load of 20 μg, except in those cases when an overloaded sample (150 μg protein) altered the results.

The plasma lipoprotein values for all patients analyzed are included in **Table 1.** Patients with type I hyperlipoproteinemia (N = 10) could be classified into two groups. One group had marked hypertriglyceridemia (mean triglyceride 2843 mg/dl, Table 1) and had both B-100 and B-48 in the density < 1.006 g/ml lipoproteins (**Fig. 1A**). The ratio of B-100 to B-48 in the electrophoretogram of the apolipoproteins of density < 1.006 g/ml was 9:1. The second group of patients had a mean triglyceride concentration of 859 mg/dl, and only B-100 with no B-48 in the density < 1.006 g/ml fraction (Fig. 1B). B-100 was the only apoB protein in IDL and LDL in both groups of patients (Fig. 1A, B).

Patients with type IIA (N = 10) and type IIB (N = 10) hyperlipoproteinemia had only B-100 in all lipoprotein density fractions as illustrated in **Fig. 2A, B.** Three patients with homozygous familial hypercholesterolemia were analyzed, and no B-48 could be detected in any density fraction (Fig. 2C).

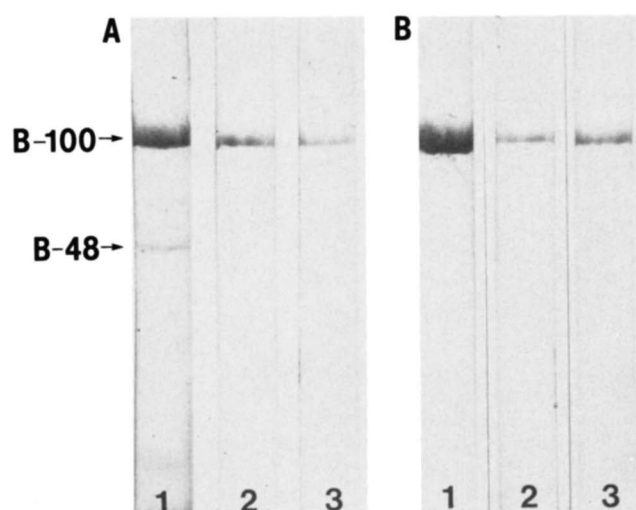Type III hyperlipoproteinemia is a disorder characterized by hypercholesterolemia, hypertriglyceridemia,

Fig. 1. Comparative analytical NaDodSO$_4$ gel electrophoresis of apoprotein B. (A) Type I hyperlipoproteinemia with high plasma triglycerides. (B) Type I hyperlipoproteinemia with low plasma triglycerides (see Table 1). 1. d < 1.006 g/ml fraction. 2. IDL fraction. 3. LDL fraction.

and cholesterol-rich VLDL. The metabolic abnormality in this disorder has been proposed to be a defect in chylomicron remnant metabolism due to the presence of an abnormal E (apoE$_2$) (33, 34) or the absence of apoE (35). The density fraction < 1.006 g/ml of all ten patients with type III hyperlipoproteinemia contained

TABLE 1. Plasma lipid and lipoprotein cholesterol concentrations

| Patient Classification	Plasma Cholesterol	Triglycerides	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol
			mg/dl		
Type I with B-48 in VLDL (N = 7)	259.5 ± 48.0[a]	2843.3 ± 1534.9	234.8 ± 51.9	17.5 ± 4.3	7.2 ± 5.8
Type I without B-48 in VLDL (N = 3)	240.7 ± 89.9	858.7 ± 403.8	198.3 ± 96.6	29.3 ± 8.1	13 ± 6.6
Type IIa (N = 10)	330.5 ± 62.2	159.4 ± 63.6	23.7 ± 11.4	252.1 ± 62.0	54.7 ± 15.0
Type IIb (N = 10)	306.5 ± 43.6	381.5 ± 189.0	50.2 ± 23.8	211.5 ± 43.2	44.8 ± 15.8
Type IIa (homozygous familial hypercholesterolemia) (N = 3)	571.0 ± 28.0	76.5 ± 18.5	38.3 ± 20.8	488.5 ± 38.5	44.5 ± 22.2
Type III (N = 10)	328.4 ± 77.4	529.8 ± 237.5	159.8 ± 87.9	125.1 ± 31.4	40.1 ± 10.9
Type IV (N = 10)	240.9 ± 36.2	410.3 ± 115.0	65.4 ± 20.9	140.2 ± 36.8	35.3 ± 7.1
Type V with B-48 in VLDL (N = 6)	425.8 ± 123.9	2661.3 ± 1530.4	365.8 ± 140.4	42.3 ± 23.5	17.7 ± 5.8
Type V without B-48 in VLDL (N = 4)	249.9 ± 32.4	696.0 ± 173.9	155.5 ± 29.7	70.3 ± 5.6	24.0 ± 1.8
Betasitosterolemia	224	161	24	120	80
Wolman's disease	181	225	24	149	8
LCAT deficiency	163	687	66	95	2
Tangier disease	36	245	23	13	0
Niemann-Pick disease	258	309	43	186	29

[a] Values are mean ± SD.
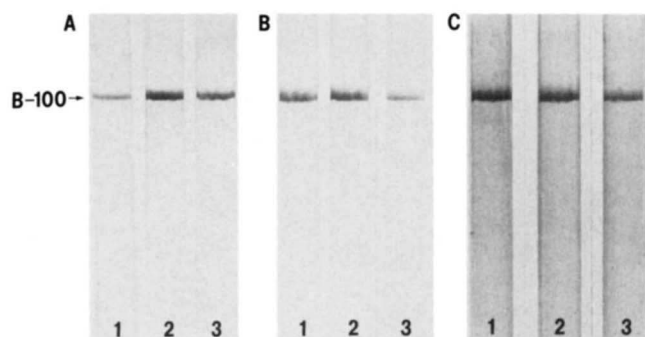
Fig. 2. Electrophoretograms of apoprotein B in 3% polyacrylamide gel. (A) Type IIa hyperlipoproteinemia. (B) Type IIb hyperlipoproteinemia. (C) Type IIa phenotype, homozygous familial hypercholesterolemia. 1. d < 1.006 g/ml fraction. 2. IDL fraction. 3. LDL fraction.

Fig. 4. Electrophoretograms of apoprotein B of type IV hyperlipoproteinemia. 1. d < 1.006 g/ml fraction. 2. IDL fraction. 3. LDL fraction.

both B-100 and B-48 at a ratio on the electrophoretogram of 10:1. B-100, however, was the only B apolipoproteinemia in IDL and LDL in nine of the ten patients. The patient with apoE absence (33), however, was unique and contained B-48 also in IDL and LDL (**Fig. 3B**). In this one patient the ratios of B-100 to B-48 in the lipoproteins of density fraction < 1.006 g/ml, IDL, and LDL were 2:1, 2:1, and 4:1, respectively.

Fig. 4 contains the electrophoretograms of the density fractions of patients with type IV hyperlipoproteinemia. B-100 was the only B apolipoprotein in all density fractions.

Patients with type V hyperlipoproteinemia can be divided into two groups, as was the case in patients with type I hyperlipoproteinemia based on B apolipoprotein composition and the degree of hypertriglyceridemia. Protein loads of 150 μg were required to demonstrate
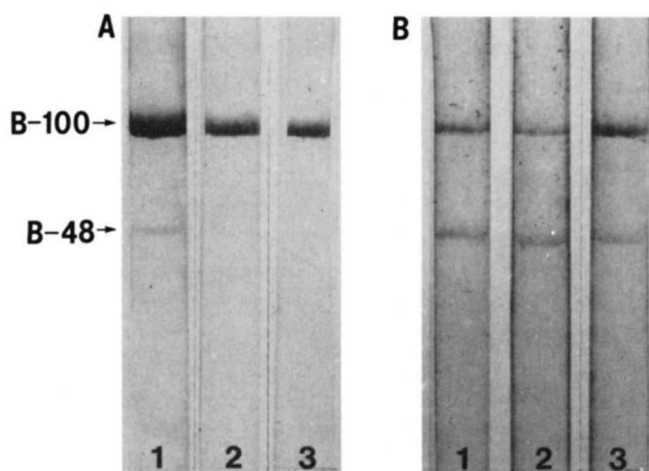
B-48 in the density fraction < 1.006 g/ml. One group (N = 6) had mean triglycerides of >2661 mg/dl (Table 1) and both B-100 and B-48 in the density fraction < 1.006 g/ml (ratio B-100 to B-48: 10–12:1), however, B-100 was the only B apolipoprotein in IDL and LDL (**Fig. 5A**). The second group (N = 4) had mild hypertriglyceridemia, a mean triglyceride concentration of 696 mg %. (Table 1), and only B-100 in all density fractions (Fig. 5B).

Fig. 3. Electrophoretograms of apoprotein B in 3% polyacrylamide gel. (A) Type III hyperlipoproteinemia. (B) ApoE deficiency and type III hyperlipoproteinemia. 1. d < 1.006 g/ml fraction. 2. IDL fraction. 3. LDL fraction.
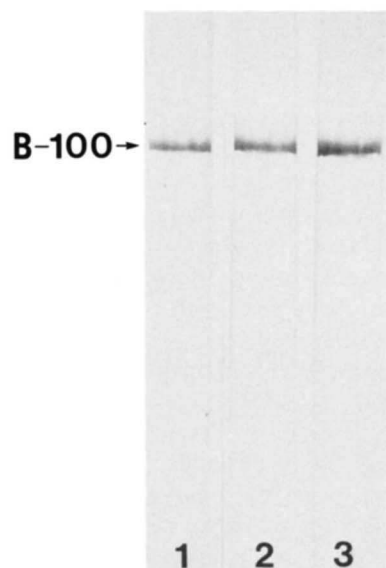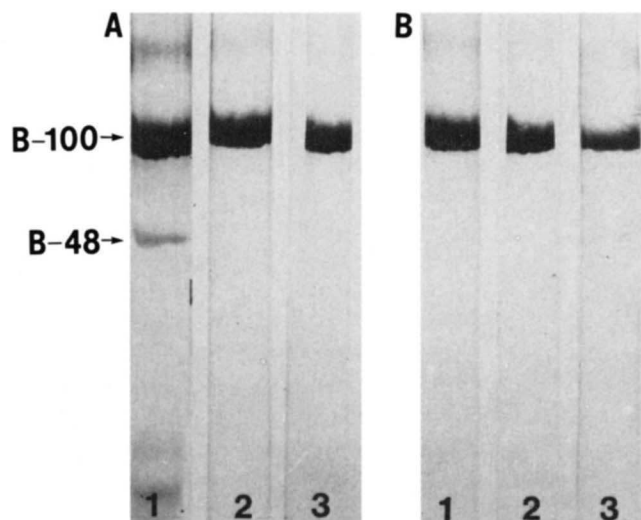

Fig. 5. Electrophoretograms of apoprotein B in 3% polyacrylamide gel. (A) Type V hyperlipoproteinemia with high plasma triglycerides. (B) Type V hyperlipoproteinemia with low plasma triglycerides (see Table I). 1. d < 1.006 g/ml fraction. 2. IDL fraction. 3. LDL fraction.

In **Fig. 6** is illustrated the analysis of the density < 1.006 g/ml fraction of patients with betasitosterolemia, Wolman's disease, lecithin cholesterol acyltransferase deficiency, Tangier disease, and Niemann-Pick disease. The patient with LCAT deficiency had a small quantity of B-48 in the density < 1.006 g/ml fraction. All other patients contained only B-100 in all plasma lipoproteins.

DISCUSSION

The identification, isolation, and characterization of the plasma apolipoproteins has permitted a more definitive analysis of the mechanisms and pathways involved in lipoprotein biosynthesis, transport, and metabolism. The discovery of the two apparent molecular weight forms of human apoB, designated B-48 and B-100, has permitted lipoprotein particles to be categorized into those that are synthesized by the liver and intestine. The present studies were designed to determine the site of synthesis of the lipoprotein particles elevated in patients with hyperlipoproteinemias types I to V, and a number of rare genetic diseases.

Analysis of plasma lipoproteins and B apolipoproteins from fasting patients with type I hyperlipoproteinemia revealed the presence of B-48 in the 1.006 g/ml density fraction in severely hypertriglyceridemic patients. The relatively low percentage of B-48 in the lipoproteins of d < 1.006 g/ml is of particular interest since it was anticipated that the majority of these lipoproteins would be of intestinal origin. The major apolipoprotein, however, was B-100, suggesting that a significant fraction of the triglyceride-rich lipoproteins in type I patients in the fasting state is of liver origin.

The pattern of elevation of triglyceride-rich lipoproteins in patients with type V hyperlipoproteinemia was very similar to those observed in type I patients. B-100 was the predominate apolipoprotein and a small quantity of B-48 was detected only at very high triglyceride levels (Table 1). In type V, as well as in type I patients, the presence of B-48 in plasma lipoproteins correlated with the degree of hypertriglyceridemia and suggested that intestinal remnants, as determined by B-48 composition, occur only at high triglyceride concentrations. Two major molecular defects, lipoprotein lipase deficiency (24) and apoC-II deficiency (25), have been identified in patients with type I hyperlipoproteinemia. Several different molecular defects may be responsible for the hyperlipidemia in type V hyperlipoproteinemia. We have recently observed a high frequency of apoE₄ in patients with type V, suggesting that abnormalities in the E apolipoproteins may be important in the pathogenesis of type V hyperlipoproteinemia in some patients (36).
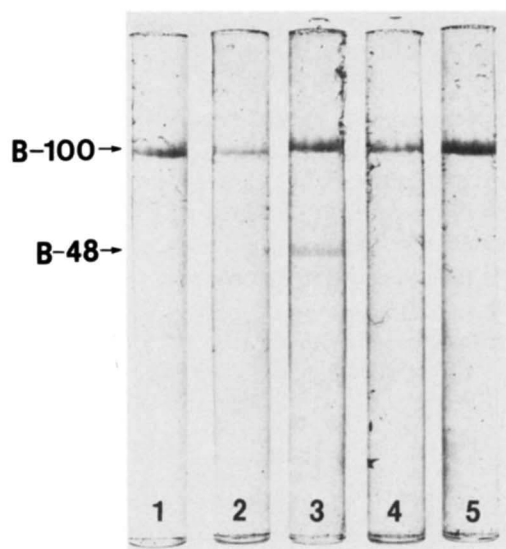

Fig. 6. Electrophoretograms of apoprotein B in d < 1.006 g/ml fraction of subjects with: 1. betasitosterolemia, 2. Wolman's disease, 3. LCAT deficiency, 4. Tangier disease, 5. Niemann-Pick disease.

Patients with type III hyperlipoproteinemia have been proposed to have a defect in chylomicron remnant removal due to a structural defect in apoE (33, 34) or apoE absence (35). The presence of B-48 in the <1.006 g/ml density fractions in type III patients is consistent with this concept. Of particular interest was the analysis of the lipoproteins from the patient with apoE absence (35). This patient was the only patient with B-48 in the 1.006 g/ml, IDL, and LDL density fractions. In this latter disorder chylomicron remnants appear to be present even within LDL.

In the patient with LCAT deficiency, a small quantity of B-48 was present in the 1.006 g/ml density fraction. This result suggests that there may be a defect in chylomicron remnant metabolism in this disorder.

The analysis of the plasma lipoproteins of patients with homozygous familial hypercholesterolemia was also of interest. Patients with familial hypercholesterolemia have striking elevations of plasma LDL due to enhanced synthesis of IDL–LDL particles and defective catabolism of LDL due to a defect in LDL receptor function (17, 37–40). The lack of B-48 in IDL–LDL in these subjects supports the concept that the increased synthesis of IDL–LDL in these patients is hepatic and not intestinal in origin.

Patients with type IIa, IIb, and IV had only B-100 in all density fractions. These results indicate that chylomicron remnant accumulation does not play a major role in these dyslipoproteinemias.

In summary, analyses of the apoB content of the plasma lipoproteins of patients with dyslipoproteinemia support the concept that B-48 is associated with tri-

glyceride-rich lipoproteins synthesized by the intestine. The degree of chylomicron and plasma triglyceride elevation in patients with type I and type V hyperlipoproteinemia was correlated with the presence of B-48 in plasma lipoproteins. Patients with types I, III, and V and the patient with LCAT deficiency have increased concentrations of B-48, suggesting a defect in chylomicron remnant metabolism. These combined results support the concept that in patients with dyslipoproteinemia the presence of B-48 in plasma lipoproteins suggests a defect of metabolism of lipoproteins of intestinal origin, and that the B-100 apolipoprotein of LDL is derived from the liver. Additional information on the metabolism of B-48 and B-100 will be obtained by a detailed analysis of the B apolipoproteins in the postprandial state, after prolonged fasting, and following diets enriched with specific dietary constituents.▐

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